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6. AUTHORS
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7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
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14. ABSTRACT
We elucidated the complete biomolecular mechanism controlling dynamically tunable reflectance from skin cells of the squid, accomplishing all major objectives of our proposed research, with potential benefit to the Army in identifying new paths for improvements in lightweight solar cells, IR detectors, and recovery of waste heat through thermal photovoltaics. Accordion-like folds in the cell membrane filled with unique reflectin proteins form the lamellae of a tunable Bragg reflector. An acetylcholine (neurotransmitter)-triggered signal transduction cascade

15. SUBJECT TERMS
Squid, Photonics; Proteins; Reflectin; Camouflage

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| UU | UU | UU | UU | | Daniel Morse |
| | | | | | 19b. TELEPHONE NUMBER |
| | | | | | 805-893-3157 |

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## Report Title

Final Report: Biomolecular Mechanisms of Adaptive Reflectance and Related Biophotonic Systems in Molluscs

## ABSTRACT

We elucidated the complete biomolecular mechanism controlling dynamically tunable reflectance from skin cells of the squid, accomplishing all major objectives of our proposed research, with potential benefit to the Army in identifying new paths for improvements in lightweight solar cells, IR detectors, and recovery of waste heat through thermal photovoltaics. Accordion-like folds in the cell membrane filled with unique reflectin proteins form the lamellae of a tunable Bragg reflector. An acetylcholine (neurotransmitter)-triggered signal transduction cascade activates catalytic phosphorylation of specific amino acids in the reflectin proteins, driving conformational changes in the proteins that activate their condensation and hierarchical assembly. The resulting occlusion of the reflectins' surface charges triggers an efflux of small ions across the lamellar membranes, subsequently inducing a Gibbs-Donnan equilibration that drives expulsion of water, shrinking the thickness and spacing of the Bragg lamellae. The result is a simultaneous increase in the intensity of reflectance and a progressive change of color of the reflected light. In related results suggesting a mechanism for improved efficiency of lightweight solar cells, we discovered that Mie-scattering from the reflectin-containing cells in Tridacnid giant clams redirects solar photons deep into the animal's tissues, increasing the efficiency of photosynthesis by endosymbiotic microalgae.

## Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

### (a) Papers published in peer-reviewed journals (N/A for none)

| Received | | Paper |
|---|---|---|
| 01/06/2015 | 10.00 | C.A. Boch, B. Ananthasubramaniam, A. M. Sweeney, F. J. Doyle III , D. E. Morse. Effects of light dynamics on coral spawning synchrony, Biological Bulletin, (06 2011): 161. doi: |
| 01/06/2015 | 11.00 | A. Ghoshal, E. Pandolfi, A. T. Weaver, D. E. Morse, M. Baum, D. G. DeMartini. Dynamic biophotonics: female squid exhibit sexually dimorphic tunable leucophores and iridocytes, Journal of Experimental Biology, (09 2013): 0. doi: 10.1242/jeb.090415 |
| 08/30/2011 | 1.00 | Amanda L. Holt, Justin G.A. Wehner, Andreas Hammp, Daniel E. Morse. Plastic Transmissive Infrared Electrochromic Devices, Macromolecular Chemistry and Physics, (07 2010): 0. doi: 10.1002/macp.201000096 |
| 08/30/2011 | 4.00 | Tao, A.R., D. G. DeMartini, M. Izumi, A. M. Sweeney, A.L. Holt and D. E. Morse . The role of protein assembly in dynamically tunable bio-optical tissues. , Biomaterials, (02 2010): 793. doi: |
| 08/30/2011 | 3.00 | Izumi, M., A. M. Sweeney, D. G. DeMartini, J. C. Weaver, M. L. Powers, A.R. Tao, T. V. Silvas, R. M. Kramer, W. J. Crookes-Goodson, L. M. Mäthger, R. R. Naik, R. T. Hanlon and D. E. Morse. . Changes in reflectin protein phosphorylation are associated with dynamic iridescence in squid, Interface - Proceedings of the Royal Society, (03 2010): 549. doi: |
| 08/30/2011 | 2.00 | A. L. Holt, A. M. Sweeney, S. Johnsen, D. E. Morse. A highly distributed Bragg stack with unique geometry provides effective camouflage for Loliginid squid eyes, Journal of the Royal Society Interface, (02 2011): 0. doi: 10.1098/rsif.2010.0702 |
| 09/03/2013 | 6.00 | A. Ghoshal, D. G. DeMartini, E. Eck, D. E. Morse. Optical parameters of the tunable Bragg reflectors in squid, Journal of the Royal Society Interface, (06 2013): 0. doi: 10.1098/rsif.2013.0386 |
| 09/04/2013 | 5.00 | D. G. DeMartini, D. V. Krogstad, D. E. Morse. Membrane invaginations facilitate reversible water flux driving tunable iridescence in a dynamic biophotonic system, Proceedings of the National Academy of Sciences, (01 2013): 2552. doi: 10.1073/pnas.1217260110 |
| 12/22/2014 | 9.00 | Zhongtao Li, Yuan Zhang, Amanda L. Holt, Borys P. Kolasa, Justin G. Wehner, Andreas Hampp, Guillermo C. Bazan, Thuc-Quyen Nguyen, Daniel E. Morse. Electrochromic devices and thin film transistors from a new family of ethylenedioxythiophene based conjugated polymers, New Journal of Chemistry, (01 2011): 1327. doi: 10.1039/c0nj00837k |
| 12/22/2014 | 7.00 | A. Ghoshal, D. G. DeMartini, E. Eck, D. E. Morse. Experimental determination of refractive index of condensed reflectin in squid iridocytes, Journal of the Royal Society Interface, (04 2014): 0. doi: 10.1098/rsif.2014.0106 |
| 12/22/2014 | 8.00 | A. L. Holt, S. Vahidinia, Y. L. Gagnon, D. E. Morse, A. M. Sweeney. Photosymbiotic giant clams are transformers of solar flux, Journal of the Royal Society Interface, (10 2014): 0. doi: 10.1098/rsif.2014.0678 |

**TOTAL:** 11

**Number of Papers published in peer-reviewed journals:**

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## (b) Papers published in non-peer-reviewed journals (N/A for none)

| Received | Paper |
| --- | --- |

**TOTAL:**

**Number of Papers published in non peer-reviewed journals:**

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## (c) Presentations

DeMartini, D.G., A. Tao, and D. E. Morse. Sept., 2009. Adaptive Bio-Optics: The Role of Dynamic Protein Assembly in Cephalopod Coloration. Biomolecular Science and Engineering Symposium, University of California, Santa Barbara,

Morse. D., Sweeney, A., DeMartini and D. Holt, A. Oct., 2009. Biological Systems Inspire New Pathways to High-Performance Materials for Energy and Photonics. Bioengineering Insights Lecture, University of California, Santa Barbara

Sweeney, A.M., Jan., 2010. Cephalopod S-crystallins, deep evolutionary time, and the origin of graded index lenses. International Congress of Eye Research

Sweeney, A.M., Holt, A.L., Mason, E., Morse, D.E. January, Jan., 2010. Deep-sea silver: photonics and biochemistry of semi-coherent broadband reflectors on squid eyes. Society for Integrative and Comparative Biology (Salt Lake City)

Holt, A. L., A. M. Sweeney and D. E. Morse. January, Jan., 2010. Optical probing and modeling of structural reflections from proteinaceous shapes in squid. Society for Integrative and Comparative Biology Annual meeting, (Salt Lake City)

Haddock, S.H.D., Figoski, L., Watts, M., Sweeney, A.M., Dunn, C.W. January, 2010. Experimental evidence for the role of fluorescent proteins (GFPs) in prey attraction. Society for Integrative and Comparative Biology (Salt Lake City)

DeMartini, D., M. Izumi, A. Andrea and D.E. Morse; January, 2010. Changes in Reflectin Phosphorylation Drives Iridescence in Squid. BioEngineering Insights Conference, Santa Barbara, CA

Morse, D.E. July, 2010. From Silica Skeletons of Sponges to Dynamically Tunable Photonics in Squid: Bio-inspired Materials Open New Horizons for Marine Biodiscovery. Marine Biodiscovery Symposium, University of Aberdeen; 7/10

Morse. D., Sweeney, A., DeMartini and D. Holt, A. July, 2010. Dynamically Adaptive Bio-inspired Photonics: Nanoscale Protein Assembly Drives Changes in Iridescence in Squid, Inspiring New Approaches to Dynamically Tunable Optical Materials. International Symposium on Nanoscale Assembly, Santorini.

Morse, D.E. Dec., 2010. Biophotonics: Variations in Nanoscale Protein Assembly Yield Wide Range of Tunable and Fixed-Wavelength Bragg Reflectors with Functions from Communication and Camouflage to Enhanced Photosynthesis International Symposium on Bio-Nanotechnology. Tokyo.

Sweeney, A., Dec., 2010. Dynamically Tunable Marine Biophotonics. Marine Science Inst., Univ. of Calif., Santa Barbara.

Sweeney, A., Dec. 2010. Dynamically Tunable Marine Biophotonics Invited talk to Applied Ocean Science Program, SIO/Univ. of Calif. San Diego seminar series, La Jolla, CA

Sweeney, A.M., Holt, A.L., Howell, A., Shaner, N.C., Morse, D.E. January, 2011. Squid reflectins and photonic self-assembly: a transcriptomic approach. Society for Integrative and Comparative Biology (Charleston, SC)

Li, Z., A. Holt, D., D. DeMartini, and D.E. Morse. February 2011. Squid-inspired tunable IR shutters. Institute for Collaborative Biotechnologies Conference. Santa Barbara, CA

DeMartini, D., A. Ghoshal, M. Izumi, A. Tao and D.E. Morse. February 2011, "Molecular Characterization of Adaptive Squid Iridescence", Institute for Collaborative Biotechnologies Conference. Santa Barbara, CA

DeMartini, D. A. Tao, M. Izumi, and D.E. Morse. April 2011, "Structure and adaptive function of squid iridophores." European Cephalopod Conference (EuroCeph 2011). Vico Equense, Italy

Sweeney, A.M., A.L. Holt, Y. Gagnon, D.E. Morse. April 2011. Bio-photonics and Monte Carlo modeling of the Tridacnid giant clam symbiosis. NASA Space Science Division meeting, Ames Research Center (Mountain View, CA).

DeMartini D. and D.E. Morse "Adaptive Structure and Function of Squid Iridophores." Biomolecular Sciences and Engineering Program Symposium. September 2011, University of California, Santa Barbara, CA

DeMartini, D., M. Izumi, A. Tao, and D.E. Morse. September 2011. "Molecular Characterization of Adaptive Squid Iridescence." Molecular, Cellular and Developmental Biology Departmental Symposium, University of California-Santa Barbara. Santa Barbara, CA

Gagnon, Y., Speiser, D.I., Sweeney, A.M., January, 2012. The visual function of the fluorescent lenses of Greeneye fish. Society for Integrative and Comparative Biology

Sweeney, A.M., Holt, A.L., Gagnon, Y., Morse, D.E. January, 2012. Giant clam iridocytes optimize photosynthetic symbiosis. Intl. Symp. Soc. Integrative and Comparative Biology (Seattle).

Holt, A.L., Gagnon, Y., Sweeney, A.M., Morse, D.E. January, 2012. A Monte-Carlo model of photon transport in symbiotic giant clams. Intl. Symp. Soc. Integrative and Comparative Biology (Seattle).

Rotstein R, Lora F, Gordon M J, Morse D E. Poster, ICB Army-Industry Collaboration Conference and Comprehensive Review; Santa Barbara; 3/12. Bio-inspired, quasi-ordered, broadband IR reflectors.

Sweeney, A. A. Holt and D.E. Morse. Oral presentation, Intl. Symp. Materials Research Soc., San Francisco. 4/12. Simultaneous Bragg reflection and Mie scattering from giant clam iridocytes facilitates photosynthesis by endosymbiotic algae.

DeMartini D. and D.E. Morse "Adaptive squid iridescence." Palau International Coral Reef Center. April 2012, Koror, Palau

Morse, D.E. May, 2012. Cellular Interfacial Architectures and Molecular Mechanisms Controlling Dynamically Tunble Color in Squid. Gordon Conference on Biointerface Science. Les Diablarets, Switzerland.

Morse, D.E. June, 2012. Dynamically Adaptive Bio-Photonics: Nanoscale Protein Assembly Drives Changes in Iridescence in Squid, Inspiring New Approaches to Dynamically Tunable Optical Materials. CIMTEC International Conference on Materials for Advnaced Technologies. Montecatini.

Morse. D.E. Invited keynote lecture, 4th International Conference on Smart Materials, Structures and Systems; Montecatini, Italy, 6/12. Dynamically adaptive bio-photonics: Nanoscale protein assembly drives changes in iridescence in squid, inspiring new approaches to dynamically tunable optical materials.

DeMartini, D., A. Ghoshal, E. Pandolfi, E. Eck, and D.E. Morse. October 2012, "Sexually dimorphoic squid iridescence offers a potential for male mimicry." Cephalopod International Advisory Council Conference. Floriannapolis, Brasil

Morse, D.E. Nov., 2012. Biologically inspired, nano-structured materials for energy and photonics. Asia-Pacific Interdisciplinary Research Conference. Irago, Japan.

Morse, D.E. Nov., 2012. Biologically inspired, nano-structured materials for energy and photonics. Symposium for Toyo University's 125th Anniversary. Tokyo.
Sweeney, A. January, January, 2013. Bartholomew Lecture: Animal Photonics, an Integrated, Comparative View. Society for Integrative and Comparative Biology (San Francisco)
Holt, A.L., Gagnon, Yl, Vahidinia, S., Morse, D.E., Sweeney, A.M. January, 2013. Photonic enhancement of symbiotic photosynthesis in giant clams. Society for Integrative and Comparative Biology (San Francisco)
Sweeney, A.M., Johnsen, S., Gagnon, Y., Morse, D.E., Stramski, D. January, 2013. Jurassic marine photonics: Squid dynamic iridescence and predation by large, extinct marine reptiles. Society for Integrative and Comparative Biology (San Francisco)
Cai, J., Heiney, P.A., Sweeney, A.M. January, 2013. Building a lens from a single protein: Small angle x-ray scattering on squid eyes. Society for Integrative and Comparative Biology (San Francisco)
DeMartini, D. A. Ghoshal, E. Pandolfi, A. Tao, and D.E. Morse. February 2013, "The Mechanism of Tuneable Squid Iridescence." Molecular, Cellular and Developmental Biology Symposium,-University of California-Santa Barbara. Santa Barbara, CA (Honorable Mention)
Sweeney, A.M, Holt, A.L, Morse, D.E., Stramski, D. March, 2013. The curvy photonics of squid camouflage. American Physical Society, March Meeting
Cai, J., Heiney, P., Sweeney, A. March, March, 2013. Material structure of a graded refractive index lens in decapod squid. American Physical Society
Morse, D.E., D. DeMartini, A. Ghoshal, A.M. Sweeney, A. Holt and A. Tao. March, 2013. Dynamically Adaptive Bio-Photonics: Nanoscale Protein Assembly Drives Changes in Iridescence in Squid, Inspiring New Approaches to Dynamically Tunable Optical Materials. 3rd Int'l. Symp. Multifunctional, Hybrid and Nanomaterials. (Sorrento)
"Dynamically Adaptive Bio-Photonics: Nanoscale Protein Assembly Drives Changes in Iridescence in Squid, Guiding New Approaches to Dynamically Tunable Optical Materials" D. E. Morse and D. DeMartini. 3rd International Conference on Multifunctional Materials. Sorrento, Italy, June 2013.
""Biophotonics: Tunable Control of Camouflage, Communication and Photosynthetic Symbiosis in Marine Molluscs"; D.E. Morse. Gordon Research Conference on Molecular Marine Ecology, Hong Kong, China, August, 2013.
"Tunable Bio-Photonics: Protein Assembly Drives Changes in Iridescence of Squid, Inspiring New Approaches to Dynamically Tunable Optical Materials"; D.E. Morse. International Symposium on Functional Materials. Sept. 2013, Rome,
"Biophotonic System Provides a Blueprint for More Efficient, 3-Dimensional Solar Cells and Solar Fuel Production"; D.E. Morse, A, Holt and A. Sweeney. International Symposium on Nano-Energy; London, December, 2013.
"Tunable Bio-Photonics: Nanoscale Protein Assembly Drives Changes in Iridescence and Enhanced Photosynthesis in Molluscs." D.E. Morse; International Photonics Conference, Duke University; December, 2013.
"Biophotonic System Provides a Blueprint for More Efficient, 3-Dimensional Solar Cells and Solar Fuel Production"; D.E. Morse, A, Holt A. Ghoshal and M. Gordon. International Symposium on Nano-Structured Materials; Houston, May, 2014.

**Number of Presentations:** 45.00

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## Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

| Received | Paper |
|---|---|

**TOTAL:**

**Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

## Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

**TOTAL:**

**Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):**

## (d) Manuscripts

Received Paper

**TOTAL:**

**Number of Manuscripts:**

## Books

Received Book

**TOTAL:**

Received Book Chapter

**TOTAL:**

## Patents Submitted

US 2011-0164303. 1/10/12. Polymer Shutter Compositions and Devices for Infrared Systems, D.E. Morse, Amanda Holt, Justin Wehner and Andreas Hampp – University of California and Raytheon Vision Systems Inc.

## Patents Awarded

## Awards

A. Sweeny awarded Bartholomew Prize, Intl. Soc. Integrative and Comparative Biology, 1/13
D. Morse elected Fellow, Materials Research Society, 4/13

## Graduate Students

| NAME | PERCENT_SUPPORTED | Discipline |
|---|---|---|
| Daniel DeMartini | 0.22 | |
| **FTE Equivalent:** | **0.22** | |
| **Total Number:** | **1** | |

## Names of Post Doctorates

| NAME | PERCENT_SUPPORTED |
|---|---|
| **FTE Equivalent:** | |
| **Total Number:** | |

## Names of Faculty Supported

| NAME | PERCENT_SUPPORTED |
|---|---|
| **FTE Equivalent:** | |
| **Total Number:** | |

## Names of Under Graduate students supported

| NAME | PERCENT_SUPPORTED |
|---|---|
| **FTE Equivalent:** | |
| **Total Number:** | |

**Student Metrics**
This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: ...... 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense ...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:...... 0.00

## Names of Personnel receiving masters degrees

| NAME | |
|---|---|
| **Total Number:** | |

## Names of personnel receiving PHDs

| NAME | |
|---|---|
| Daniel DeMartini | |
| **Total Number:** | **1** |

## Names of other research staff

| NAME | PERCENT_SUPPORTED |
|---|---|
| Mary Baum | 0.21 |
| **FTE Equivalent:** | **0.21** |
| **Total Number:** | **1** |

## Sub Contractors (DD882)

## Inventions (DD882)

## Scientific Progress

## Technology Transfer

# ARO FINAL REPORT

**Daniel E. Morse**
**December 10, 2014**

**Proposal # 57431-LS**

**Agreement # W911NF-10-1-0139**

**Title: "Biomolecular Mechanisms of Adaptive Reflectance and Related Biophotonic Systems in Molluscs"**

**Program Manger: Dr. Stephanie A. McElhinny**

**Reporting period: 6/1/10-8/31/14**

## Scientific Objectives and Accomplishments:

### Scientific Objectives:

Our objective was to discover and harness the previously unknown molecular mechanisms that govern the remarkable capabilities for dynamically tunable optical camouflage and intraspecies communication in squid as a model for new materials and modalities for optoelectronic communication. The immediate goals of this research were thus: (1) to identify the biomolecular and biophysical mechanisms responsible for neuronally activated changes in reflectin protein modification, conformation and assembly; and (2) to discover how these processes, in conjunction with other biomolecular and biophysical mechanisms, drive the dynamically tunable changes in the intensity and color of light reflected by the iridocyte cells in squid skin.

Our long-term aim, beyond the scope of this present and insufficiently funded proposal, is to use the information obtained from this project to design and synthesize materials that translate the discovered biophotonic mechanisms into practical engineering, chemistry and physics. (Such translation of biomolecular mechanisms underlying the synthesis and performance of biomolecular materials has been the theme and motivation for my research for the past 2 decades, as exemplified first in my discoveries with the abalone shell, and then with the mechanism of biosilica synthesis, translating these to new routes for semiconductor synthesis.

### Accomplishments with Highest Importance and Relevance to Army Needs:

We successfully achieved all of the major objectives as initially proposed.

Cephalopods (e.g., octopus, squid and cuttlefish) manipulate light for camouflage and inter-individual signaling through a combination of tunable light scattering, pigmentation and reflection [1, 2]. The tunable reflectors responsible for the dynamic iridescence of the cephalopod's skin are comprised of proteins called reflectins [3]; these are organized within stacks of intracellular, membrane-enclosed Bragg reflectors that in some contexts act as filters and in other contexts as reflectors [1-7]. In research supported by this grant and the Army-supported ICB over the past year, we discovered the complete molecular and biophysical mechanism driving this tunable and switchable biophotonic process, and confirmed that it can serve as the basis for a new approach to tunable and switchable polymer-based filters in the IR.

We discovered the complex, multi-step sequential process controlling the dynamically tunable biophotonic performance of the reflective iridocyte cells in squid skin: We found that the acetylcholine (neurotransmitter-triggered), signal transduction cascade-activated catalytic phosphorylation of specific amino acids in the reflectin proteins drives conformational changes in the proteins that activate their hierarchical assembly, simultaneously tuning the spacing, thickness and density (refractive index) of the thin protein layers and quickly driving changes in the transmission of light across the entire visible range [1, 2, 4, 5, 7]. The components and properties giving this biological system its remarkable and unique tunability of reflectance are the biopolymer's inherent elasticity, conformability, and capacity for rapid and reversible assembly and disassembly, and the synergistic effects of changing both the density (i.e., refractive index) and thickness of the layers – thereby tuning both the intensity and color of the reflected light. Details are described below. Applications of these findings to improvements in lightweight solar cells, IR detectors and recovery of waste heat through thermal photovoltaics are currently under active investigation via the ICB, in close collaboration with colleagues at the ARL.

**<u>Details of the Biomolecular and Biophysical Mechanisms of Tunable Biophotonics</u>:**

The complete molecular mechanism governing the dynamically tunable reflectance in these cells is illustrated schematically in **Figure 1** [redrawn from our publication [7] by the Editor of *The Scientist* [8], who selected our discovery and publication for special commendation]: In response to optical stimuli, release of the neurotransmitter, acetylcholine, (ACh,), from nerve endings ending in discrete patches of the skin, activates a muscarinic ACh receptor that in turn activates a receptor-coupled G-protein; this then activates a phospholipase- and calcium-controlled intracellular cascade of enzymatic amplifications of the stimulatory signal, culminating in activation of the enzymatic phosphorylation of specific reflectin proteins – the unique constituents of the tunable Bragg reflectors in squid skin [4, 5, 7]. *Our two major discoveries this past year, allowing us to fully understand the molecular mechanism of optical tuning,* are the facts that the Bragg Lamellae are formed by repeated invaginations of the cellular membrane, thus providing a high surface area interface between all parts of the tunable photonic structure and the external environment, and that this high surface area facilitates the rapid dehydration and rehydration of the Bragg lamellae, tuning their thickness and spacing to tune the wavelength of their reflected light [7]. We discovered that the signal-activated enzymatic phosphorylation of the reflectin proteins effectively neutralizes the excess positive charge carried by these proteins, thus overcoming their Coulombic, electrostatic charge repulsion, and permitting pi-pi interactions to now drive condensation of the proteins. This condensation of the reflectin proteins masks charges on the protein surfaces, causing a rapid efflux of small ions across the Bragg lamellar membranes to maintain electrostatic neutrality, which in turn causes a rapid and pronounced Gibbs-Donnan efflux of water from the Bragg lamellae to maintain osmotic equilibrium [7]. We accurately measured the reversible efflux of water from the Bragg lamellae, and the subsequent influx during the recovery of transparency, using $D_2O$ as a tracer (7).

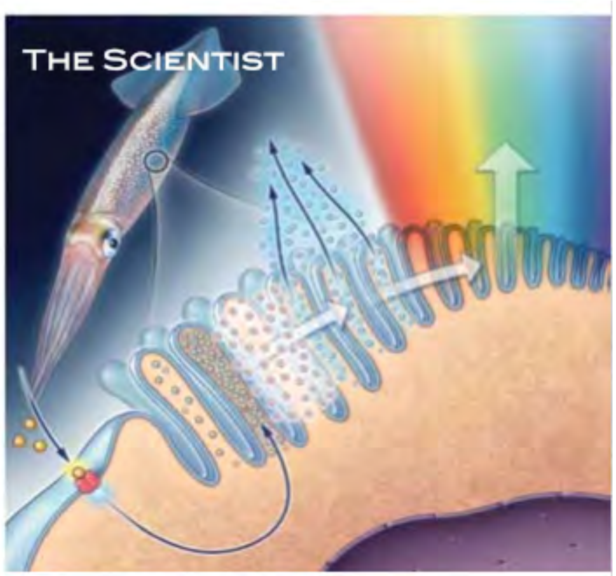


**Figure 1.** Complete molecular mechanism governing the dynamically tunable reflectance in squid skin cells, as described in detail above. From [7] and [8].

It is these changes (condensation of the reflectin proteins and consequent dehydration of the lamellae) that cause the rapid increase in the refractive index of the Bragg lamellae and the simultaneous decrease in the thickness of these Bragg reflectors, while simultaneously increasing the spacing between them (**Figure 1**) [5, 7]. This synergistic interaction thus rapidly increases the intensity of reflection and progressively changes the color of the reflected light – as we have quantified both microscopically and spectrophotometrically [2, 9, 10].

Using a micro-spectrophotometer of our own design, we independently measured the photonic parameters of the tunable Bragg lamellae as a function of their progressive condensation in the living reflective cells, and from these measurements obtained the first measures of the Bragg parameters independent of electron microscopy (requiring fixation and shrinkage of biological tissues) (**Figure 2**) [9, 10].

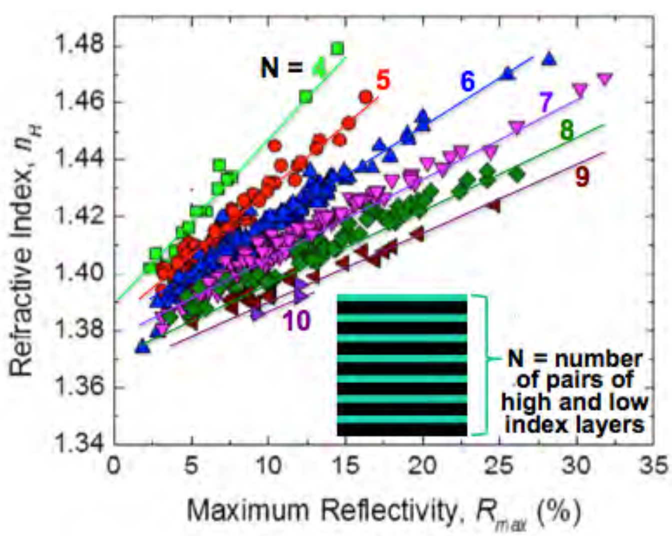


**Figure 2.** Maximum reflectivity of live squid skin cell Bragg lamellae as a function of the refractive index of the lamellae, measured with the micro-spectrophotometer developed in our lab. Data sorted by the measured number of lamellae in each reflector analyzed. From [9].

Results of these analyses (**Figure 2**) show that the tunable reflectors typically consist of 4-10 coherent Bragg lamellae, with the refractive index of the reflectin-filled lamellae in the living reflective cells progressively increasing from 1.34 in the "off" (transparent) state to 1.48 in the fully condensed and maximally reflective state. As expected, brightness is proportional to the refractive index contrast, and – as proof of the exceptional resolution of this method – at any given refractive index (i.e., state of condensation of the reflectin proteins), the contribution to brightness is greatest for those Bragg reflectors

containing the most lamellae (**Figure 2**). These results also demonstrate that the complete tuning of reflected color from red to blue is driven by the progressive reduction of the lamellar thickness of ca, 115 nm (avg.) for the high-index reflectin-containing lamellae and ca. 85 nm (avg.) for the low-index inter-lamellar spaces [9,10]. These wholly independent optical measurements also unequivocally show for the first time that prior measurements estimated from electron microscopy of dehydrated specimens were in error by ca. 25% as the inevitable result of shrinkage prior to imaging.

**<u>Discovery of a Unified Mechanism</u>*:***

We discovered that female squid of the species we studied contain cells that can quickly switch from transparency to bright white reflectance [11]. These cells (named leucophores), contain the same reflectin proteins, neurotransmitter receptors and switchable signal transduction activators controlling reflectin condensation as we discovered in the tunable iridocytes discussed above, but the reflectins are organized in thousands of small, spherical vesicles, rather than in the Bragg lamellae formed by invaginations of the cell membrane as found in the tunable color-reflecting cells. As a result, ACh-activation of the condensation of the reflectins in these cells, and the consequent dehydration and change in volume and dimension of the reflectin-containing vesicles, results in a dramatic increase in the refractive index of the vesicles and – by virtue of their size and shape - the onset of Mie-scattering of all wavelengths, thus producing omnidirectional broadband reflectance of bright white (much like the metal oxide nanoparticles in white paint). The reversible efflux of water is apparently facilitated by membrane conduits that we observed to communicate from each vesicle to the cell's exterior [11]. Using the micro-spectrophotometer described above, we determined that the refractive indices of these reflectin-containing vesicles in their transparent and white reflective states are identical to those in the corresponding states of the Bragg lamellar reflectors, as is the mechanism of their reflectin-mediated photonic control. While the molecular mechanism is the same in both types of reflective cells, the morphologies and dimensions of the dehydrated vesicles dictate that omnidirectional, broadband Mie scattering dominates their photonic behavior, yielding bright white reflectance. A comparison of the tunable color and switchable white reflective systems in their corresponding transparent and reflective states is shown in **Figure 3**.

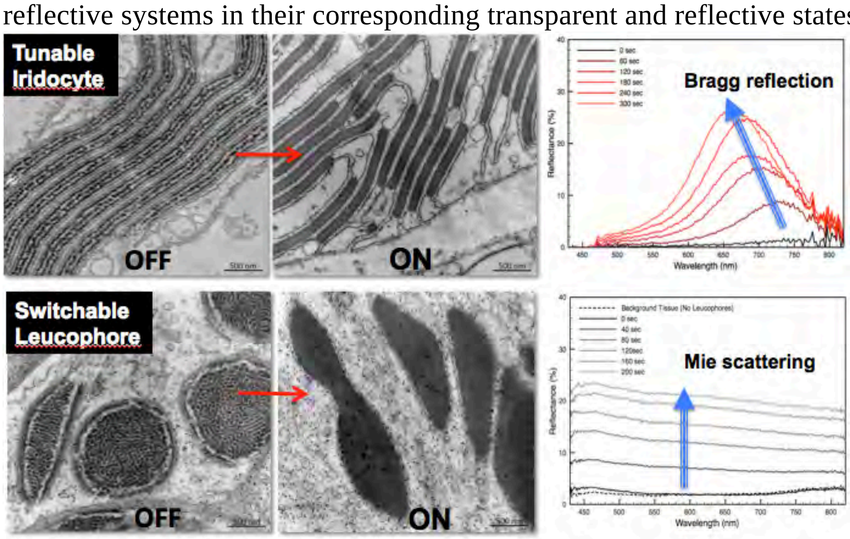


**Figure 3.** Unified molecular and biophysical mechanism controlling both the tunable color and the switchable bright white reflectance in the squid skin iridocytes and leucophores. Although the underlying molecular mechanism driving both systems is the same, and governed by the same ACh neurotransmitter, reflectin proteins, and reflectin-governed dehydration, the mode of reflection (Bragg reflection or Mie scattering) and the visible consequences differ as a result of the differences in membrane architectures enclosing the reflectins. From [11].

**<u>New Biological Inspiration For Higher Efficiency, Lightweight Solar Cells</u>*:***

The efficiency of present solar cell materials and devices is limited by the efficiencies of photon capture and useful transduction; these in turn are limited in part by the depth of penetrance of solar photons in the absorptive, energy transducing material, thereby limiting the useful thickness of such materials to thin films. In contrast, we discovered, the light-exposed epithelium of tropical giant clams of the genus *Tridacna* (that depend for their nutrition on photosynthesis performed by symbiotic microalgae living

within their tissues) contains a layer of bifunctional, bidirectionally reflective cells that use Mie scattering to deliver photosynthetically productive wavelengths of light deeper and laterally into the tissue of the host organism, while simultaneously using Bragg reflection to back-reflect non-productive wavelengths.

Giant clams (genus *Tridacna*) derive much of their nutrition from photosynthesis by their endosymbiotic microalgae (genus *Symbiodinium*), yet the typical midday solar irradiance in their shallow tropical Pacific environment (ca. 1700 µmol quanta $m^{-2}$ $s^{-1}$) far exceeds the ca. 100 µmol quanta $m^{-2}$ $s^{-1}$ threshold for photo-inhibition in their algal symbionts. We discovered [12] that a layer of brightly reflective iridocyte cells in the Tridacnid's mantel epithelium, through a combination of Mie-scattering and Bragg reflection, scatter the most photosynthetically productive wavelengths forward and laterally deeper into the clam tissue, redistributing this light onto the high surface area of vertically oriented pillars of the microalgae, while back-reflecting less productive wavelengths. Results of our direct radiometric measurements are confirmed by computational photonic modeling by Discrete Dipole Analysis, computation of the Henyey-Greenstein phase function, and Monte Carlo modeling of radiative transfer in this complex photonic system [12].

The measured high-efficiency redistribution of solar flux from the iridocyte layer to uniform illumination of the ca. 10-times higher surface area of the vertical algal pillars allows maximal capture efficiency of the solar photons, while stepping-down the incident flux on the algae by ca. 10-fold ensures maximal photosynthetic efficiency close to the algal optimum irradiance of ca. 85 µmol quanta $m^{-2}$ $s^{-1}$. We determined that the resulting photodynamic tolerance and efficiency are far higher than possible without the redirection of light by these unique iridocytes.

The results of the unique system of wavelength-selective forward scattering that we discovered in giant clams are illustrated in **Figure 4**.

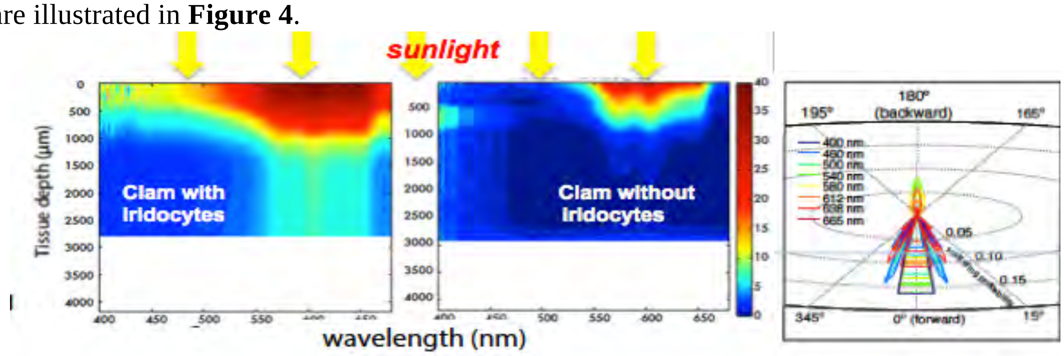


**Figure 4.** Enhancement of solar photon penetrance and capture in the biological "3-D solar cell" discovered in giant clams. (Left & center:) Scalar irradiance (total light intersecting a point from all directions) in the presence and absence of iridocytes. (Right:) Discrete Dipole Approximation-predicted phase function for the iridocyte Mie-scattering cells, on radial coordinates, showing forward-cone scattering behavior predicted for iridocytes. These data show significant back scattering for yellow light, but only forward scattering in red and blue, corresponding to our measurements. From [12 ].

As shown here, direct radiometric measurements as a function of depth into the clam tissue [12] (**Figure 4,** left and center) reveal that forward Mie scattering from the reflective cells (iridocytes) projects photons deeper and laterally into the tissue, enabling a greater volume of algal cells to perform photosynthesis than in the absence of the iridocytes. Calculations of the angle- and wavelength dependence of this forward scattering using the Henyey-Greenstein phase function [12] **(Figure 4**, right) confirm that the reflective cells direct photosynthetically productive wavelengths downward and laterally onto the high surface area of the vertical algal pillars. These results are quantitatively confirmed by our independent predictions calculated from Lorenz-Mie Theory and Discrete Dipole Analysis [12]. The key to this high-

performance system is two-fold: (1) the unique trajectories of the Mie-scattered photons (**Figure** 4), and (2) the unique arrangement of the photosynthetic algae in “vertical” micropillars within the clam tissue, optimizing their interaction with the photosynthetically productive wavelengths that are scattered by the reflective cells deep and laterally into the tissue. This unique vertical arrangement and 10-fold increase in photon-capturing surface area of the transducing algae results in the illumination of a higher density of useful solar transducers, and a higher efficiency in solar photon capture and photon transduction. Indeed, our micron-scale intra-tissue radiometry and optical modeling show that this biophotonic system results in a five-fold increase in photons reaching the microalgae compared to the tissue lacking the reflective cells [12]. This highly evolved “3-dimensional” biophotonic system of giant clams thus suggests a strategy and blueprint for more efficient, photo-bleaching-resistant solar cells and more spatially efficient solar production of algal biofuels.

**Transition in Collaboration with ARL and Raytheon:**

Biology thus shows us that these exquisitely finely-tuned lightweight polymers (proteins), when appropriately constrained in microstructured thin layers, can tune all the optical functionalities required by the Army for the applications to IR sensors and power generation conventionally provided by heavy, bulky, and in some cases noisy and power-hungry devices. Guided by this biological inspiration, we have begun to transition the lessons learned from this research, in work supported through the ICB, and in collaboration with colleagues at ARL (SEDD) and Raytheon. to develop and optimize a family of synthetic polymeric thin films that exhibit electrically driven simultaneous changes in morphology and refractive index. The lesson we deduce from the unified biological mechanism described above is that the synergistic interaction of two or more drivers of the optical change (i.e., the simultaneous change in refractive index contrast and morphology is more effective than any single change. Translating this biological inspiration to device fabrication, in collaboration with our long-time colleagues at nearby Raytheon Vision Systems, we combined the effects of a Fabry-Perot cavity (increasing the effective path-length or effective thickness of our optical polymer) with the electrically switchable changes in absorption of our engineered electrochromic polymers, to achieve an exponential gain in responsiveness to small applied voltages [13, 14] Other potential applications currently under active investigation with colleagues at ARL SEDD include improved efficiency of lightweight solar cells and recovery of waste heat through higher efficiency IR (thermal) photovoltaics.

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